Studies on Sharks – Ⅶ*.

Reproduction in Female *Mustelus griseus*

By

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It has been, heretofore, considered that Japanese *Mustelus* (Triakidae) consists of 3 species: *M. manazo* BLEEKER, *M griseus* PIETSCHMANN and *M. Kanekonis* (TANAKA)[1),2)]. The present authors[3)] have, however, suggested that 2 species, *M. manazo* and *M. griseus*, form the Japanese *Mustelus*, because *M. kanekonis* is considered to be a synonym of *M. griseus*. The comparison of reproduction in these 2 species reveals that *M. manazo* belongs to viviparous non-placental type and *M. griseus* viviparous placental[3)]. In the present investigation, the reproduction, including from ovulation to parturition, of *M. griseus* has been carried out.

Material and Method

Thirty-eight specimens of female *Mustelus griseus* were obtained from the fish market in Shimonoseki, Japan, during the period June, 1972 to April, 1973. The sharks were mainly caught by bull trawlers in the East and South China seas. The specimens were measured, external characteristics observed and gonads preserved in 10% formalin solution. The observation was carried out by macro anatomy.

Observation and Discussion

1. The female reproductive system

The selachians have a pair of organs called epigonal. Ovary in female and testis in male are formed at the anterior ends of the epigonal organs. The epigonal organs in both sexes show the quite similar histological figure[6)].

In *Mustelus griseus*, when the individual is immature, the right and left epigonal organs are slender and small in shape. As the shark grows, the left epigonal organ becomes

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rudimentary. The right one, however, continues to grow, and the ovary is made at its anterior end; only the right ovary is functional. This is also observed in *M. manazo*. On the other hand, both ovaries are functional in the species of *Squalus*[7),8)] and *Scoliodon*[9)].

In the ovulating season, June and July, the 5–16 mature eggs of about 15 mm in diameter are ovulated; the eggs are received through common ostium and distributed to both oviducts. The eggs are moved towards nidamental gland where they are fertilized. The fertilized eggs are enclosed in the brownish, transparent embryonic membrane in the nidamental gland and descend into the uterus to develop into the embryos.

2. Uterine compartments

The uterine compartments are made, as the embryos grow, according to the number of embryo. The formation of compartments in *Mustelus griseus* appear to be a little different from that of *Sphyrna tiburo*[10)]. The elevations or ridges of internal surface of the uterine wall wrap the embryos and yolk sacs from their both sides and overlap to form the compartments, but the ridges at the overlapping portion are not fused. The embryonic membrane attaches to the internal surface of the compartments, and this membrane is considered to prevent the overlapping portion of compartment from separating.

The uterine compartments are completely formed when the embryos have attained the total length of about 100 mm. At this stage, the uterine ridges at the overlapping portion of compartments appear to be completely fused. The overlapping portion of uterine compartment containing the embryo of about 280 mm was, however, separated. Re-examining the uterine compartments having the embryos of about 150 mm reveals that the compartments are not formed by the fusion; at this stage, overlapping portion is extensive, and there, uterine ridges are tightly attached each other. As the embryos grow, the overlapping portion becomes narrower and separated; then, parturition occurs.

The uterine compartments are formed when the embryos are about 100 mm in total length. At this stage, compartments are positioned obliquely. When the embryos grow more than 150 mm in total length, the compartments become more longitudinally positioned. This change in the position is considered to be due to the increase in body length of the embryo, and to make parturition easy[3)].

The ridges of internal surface of the uterine wall have been observed when the fertilized eggs are present in the uterus; the eggs are separated by the ridges (Figs. 1 and 2 in Plate I). At this stage, the ridges are already positioned obliquely and then increase in height to form the uterine compartments.

The uterine compartments are made not only in the placental species, but also in non-placental such as *Mustelus manazo*[3),5)], *M. vulgaris*, *Galeus canis* and *Galeorhinus japonicus*[11)]. The formation of compartments in *M. manazo* is similar to that of *M. griseus*. But the wall of compartment is thinner than that of *M. griseus*.

The position of compartments in the uteri containing 2 to 5 embryos is schematically shown in Fig. 1. This figure indicates that all the compartments are not formed at the same position, but a little different in height; in transverse sections, the compartment 2 is

positioned a little higher than the compartment 1, and the compartment 3 is higher than the compartment 2. The embryo of the lower positioned chamber is usually a little longer

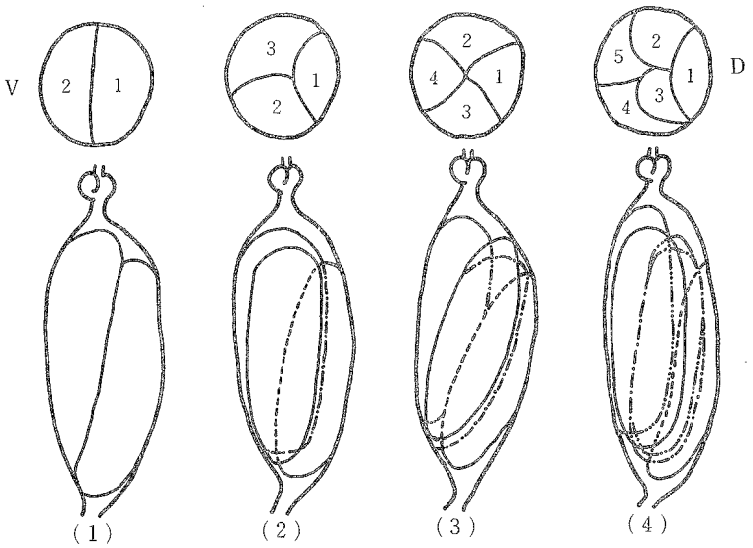

Fig..1. Position of uterine compartments in *Mustelus griseus*, upper: transverse section of uterus, lower: longitudinal section, D: dorsal side of uterus, V: ventral side. (1) adult: 945 mm, embryos: 140 and 151 mm, (2) adult: 868 mm, embryos: 235, 236 and 237 mm, (3) adult: 901 mm, embryos: 112, 118, 119 and 124 mm, (4) adult: 951 mm, embryos: 141, 143, 147, 150 and 152 mm in total length.

in total length than that of the higher. The lowest positioned embryo appears to be first born.

3. The embryonic membrane in uterus

Several fertilized eggs were fonud in the uteri of females caught in July. Each is enclosed in an embryonic membrane (Figs. 1 and 2 in Plate I). The embryonic membrane encloses the embryo until parturition. The protion of membrane not enclosing the egg is enfolded in width of about 5 mm at its both sides. (Fig. 2 in Plate I). This is also observed in *Mustelus manazo*[5].

In *Mustelus griseus*, as gestation advances, the free ends of embryonic membrane at both sides of the fertilized eggs are wrapped by the folds of internal surface of uterus, and deposited in the chamber formed by the uterine folds; this is similar to the embryonic membrane-store chamber observed in *Carcharhinus dussumieri*[4].

In the uteri of 2 females which contained the fertilized eggs, 2 cases were found; in the 1st case, the free ends of embryonic membrane were already wrapped by the uterine folds, and in the 2nd case, all the portion of membrane was still present. This suggests

that the chamber in *Mustelus griseus* should be formed in early gestation. The embryonic membrane in the chamber is deposited in folds of 5 mm in width, and the membrane passes out from the chamber as the embryo grows. One uterine compartment possesses one chamber for the embryonic membrane.

4. Establishment of the placenta

The longitudinal folds are formed in the internal surface of uterus having the fertilized eggs. At this stage, the posterior end of uterus is not yet closed.

When the embryos are 30 – 50 mm in total length (Fig. 3 in Plate I), the uterine ridges which separated the fertilized eggs increase in height and begin to wrap the embryos and their yolk sacs. The uterine folds of about 5 mm in height are observed in the internal surface of uterus. At the same time, the formation of external gill filaments of the embryo is observed. The posterior end of uterus is closed. This stage is in about the 1st and a half month of gestation.

When the embryos have attained the total length of 90 – 100 mm (Fig. 4 in Plate I), the placenta is established, and at the same time, the uterine compartments are formed. The placenta is located at the posterior end of each uterine compartment on the ventral wall of uterus[3] (Figs. 5 and 6 in Plate II). At this stage, yolk sac still contains a large quantity of yolk. The grooves in the distal surface of yolk sac and the folds in the posterior portion of the uterine compartment are interdigitated to establish the placenta. The embryonic membrane intervenes between a maternal placental portion and a foetal. The capillaries net work beneath the epithelia of these 2 placental portions are much more developed than those of the other regions. The embryos have the external gill filaments of 5 – 7 mm long. The placenta is formed when it is in about the 2nd and a half to the 3rd month of gestation.

When the embryos are about 150 mm in total length, the grooves in the surfaces of maternal and foetal placental portions become more convoluted, and these 2 surfaces are tightly interdigitated. The placenta at this stage is located between the ventral wall of uterus and the wall of compartment, and this resembles the pocket type placenta[4] found in *Carcharhinus dussumieri*. The embryos can be seen through the thin uterine wall. The uterine chambers are located longitudinally, as described before. At this stage, almost all the yolk is utilized, and the external gill filaments disappear. This stage is in about the 3rd and a half to the 4th month of gestation.

When the embryos are 240 mm in total length (Figs. 5 and 6 in Plate II), the grooves on the surface of foetal placental portion become deeper, and moreover, very small folds are formed on the surface of grooves. These very small folds are interdigitated with those of the surface of maternal portion. The foetal portion is reddish and much vascularized. The internal surface of uterine wall not participating in the placenta is extremely smooth. At this stage, as the uterine wall is very thin, the embryos in the uterus are much more visible than before.

When the embryos attain 270 – 290 mm in total length, the overlapping portion of uterine compartment is separated, and the embryo enclosed in the embryonic membrane

is exposed (Fig. 7 in Plate II). The foetal placental portion is easily separated from the maternal. These facts suggest that this stage should be just before the occurance of parturition. This stage is in about the 9th month of gestation.

After establishment of the placenta, almost all the embryos are positioned with their heads pointing anteriorly in the uterine compartments.

The embryonic membrane exists between maternal and foetal portion of placenta, even after establishment of the placenta. This is a characteristic of the selachian placenta.

The umbilical stalk connecting the embryo with a foetal placenta consists of 3 channels: umbilical artery, umbilical vein and ductus vitellointestinalis. The umbilical stalk of *Mustelus griseus* lacks the appendiculae.

5. Growth of the embryo and ovum

Ovum (Figs. 2 and 3): in *Mustelus griseus*, oocyte of about 14 mm in diameter in June, is mature. This species ovulates in June and July. The oocyte of about 4 mm in diameter in July, indicates the remaining oocyte after ovulation. The remaining oocyte then becomes smaller before September, but it becomes larger after September.

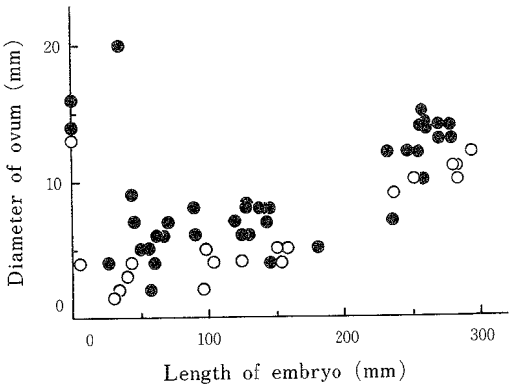

Fig. 2. Relationship between embryo length and ovum diameter in *Mustelus griseus* (open circles) and *M. manazo* (closed circles).

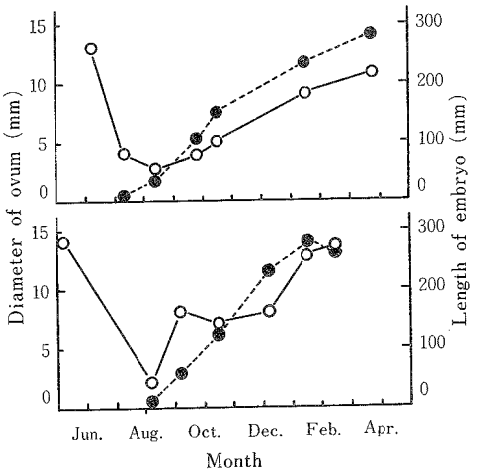

Fig. 3. Relationship between growth of embryo (closed circles) and ovum diameter (open circles) by month in *Mustelus griseus* (upper) and *M. manazo* (lower).

Although the histological observation will be carried out in the near future, at this stage, it is estimated as follows; the immature oocytes remained in the ovary after ovulation are rapidly reabsorbed. At the same time, new oocytes are developed for the succeeding ovulation. The newly developed oocytes grow with the embryonic development. They reach the maturity around next May, and are ovulated in June and July. Then, the shark starts the succeeding gestation.

This phenomenon is also observed in *Mustelus manazo* and *Carcharhinus dussumieri* [4].

Growth of embryo (Fig. 3): the fertilized eggs were found in July. It is, therefore, considered that gestation starts around this season. When the embryos attain the total length of about 100 mm around September, the placenta is established. Around October, when the embryos are 150 mm, the external gill filaments of embryo disappear, and almost all the yolk is absorbed. Around next March and April, when the embryos are 270 – 290 mm, the uterine compartments are opened, and at the same time, the foetal placental is easily separated from the maternal; parturition appears to occur in April and May when the embryos are approximately 300 mm in total length. Gestation period is, therefore, estimated to be about 10 months. The reproductive season and gestation period of *Mustelus griseus* are in accord with those of *M. canis* in the western Atlantic. The lengths of embryos at birth are also the same in both species[12].

The placenta is established when it is in about the 2nd and a half to the 3rd month of gestation. The yolk is completely absorbed when the embryos are between 150 and 200 mm in total length; this stage is in about the 4th to the 5th month of gestation. It is, therefore, considered that embryonic development of *Mustelus griseus* consists of 3 stages according to nutritional supply system; 1) the stage mainly nourished by yolk stored in the yolk sac; the stage of 2 and a half to 3 months before establishment of the placenta, 2) that by both of yolk and mother; about 2 months after establishment of the placenta, 3) that mainly nourished by mother; about 5 to 5 and a half months before parturition; at this stage, placenta is well developed. These 3 stages were also observed in *Carcharhinus dussumieri*[13].

6. Adult length and the number of embryos

The relationship between adult length and the number of embryos per litter is shown in Fig. 4. In *Mustelus griseus*, when the adults are 835 – 1023 mm in total length, the number of embryos per litter ranges between 5 and 16. In *M. manazo*, the adults being

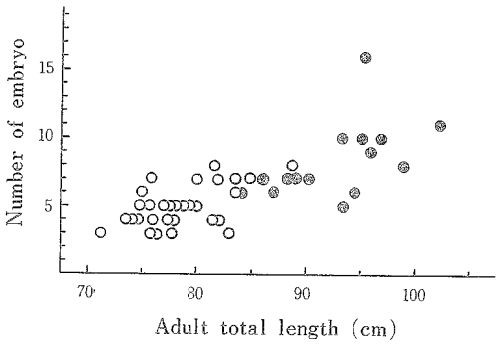

Fig. 4. Relationship between adult total length and number of embryos per litter in *Mustelus griseus* (closed circles) and *M. manazo* (open circles).

712－884 mm, it ranges 3 and 8. In both species, the number of embryos per litter increases with the increment of adult length.

In the specimen of *Mustelus griseus*, the smallest gravid female was 760 mm in total length; this shark had the fertilized eggs in its uteri. In *M. manazo*, that was 712 mm.

Summary

The reproduction, including from ovulation to parturition, of *Mustelus griseus* was investigated, and the following results were obtained;

1. Only the right ovary is functional; during ovulating season, June and July, the 5－16 mature eggs of about 15 mm in diameter are ovulated.

2. The immature oocytes remained in the ovary after ovulation are rapidly reabsorbed; at the same time, the new oocytes are considered to be developed. The newly developed oocytes grow with the embryonic development, and reach the maturity until next April and May; they prepare for the succeeding ovulation.

3. The uterine compartments are formed according to the number of embryos; one embryo enclosed in an embryonic membrane is situated in each compartment. The uterine ridges wrap the embryos from their both sides to form the compartments; they are formed when the embryos are about 100 mm in total length.

4. The free portions of embryonic membrane not enclosing the embryo and yolk sac are enfolded in width of about 5 mm in a chamber formed by the uterine folds; the membrane passes out from the chamber as the embryo grows.

5. The placenta is established, around September, when the embryos attain the total length of about 90－100 mm; it is located at the posterior end of each uterine compartment on the ventral wall of uterus. This stage is in about the 2nd and a half to the 3rd month of gestation.

6. The embryos are situated with their heads pointing anteriorly in the compartments of uterus. The number of embryos per litter ranges between 5 and 16, the adults being 835－1023 mm in total length; the number of embryos per litter increases with the increment of adult length. The smallest gravid female was 760 mm.

7. Parturition occurs in April and May when the embryos are approximately 300 mm in total length. The gestation period is about 10 months.

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PLATE

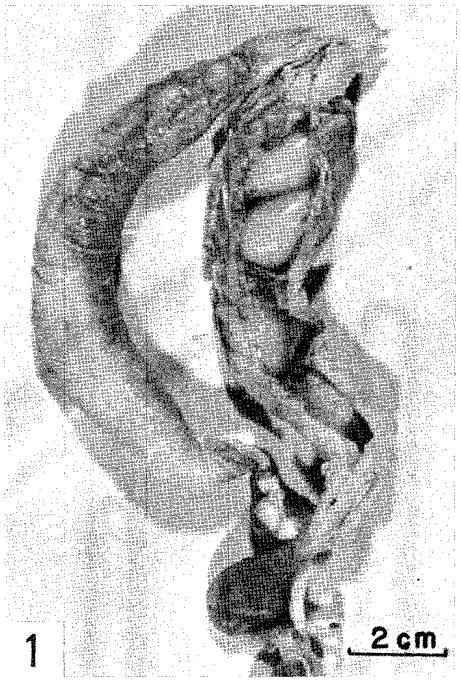
Plate I

Fig. 1. Fertilized eggs in uterus.

Fig. 2. Uterine ridges, separating fertilized eggs, are observed; each egg is enclosed in an embryonic membrane. Collected, July 20, 1972. Same uterus as Fig. 1.

Fig. 3. Uterus with 5 embryos of about 30 mm total length. Uterine ridgs increase in height. Collected, Aug. 24, 1972.

Fig. 4. Uterus with 3 embryos of 93, 103 and 104 mm. Placenta is established, and at the same time, uterine compartment formed.
Dorsal side of uterus is dissected. Collected, Oct. 3, 1972.



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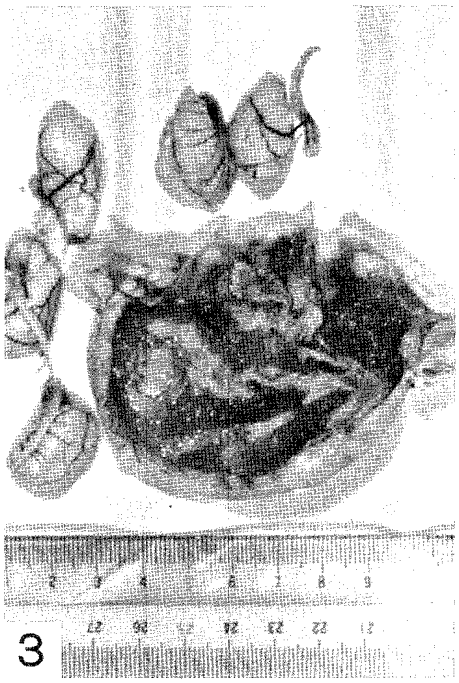

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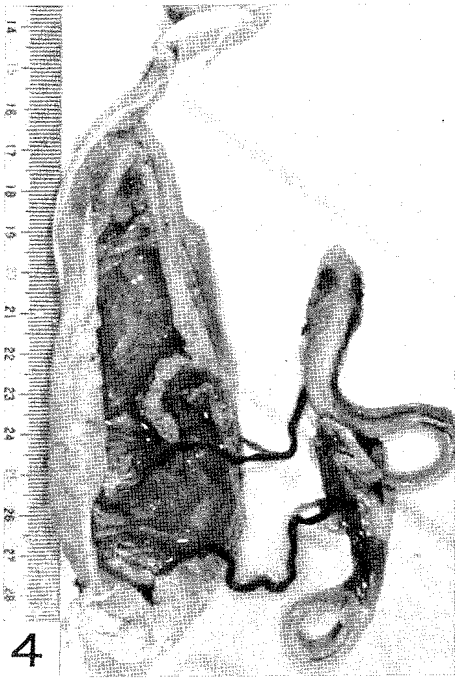

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